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On Nitrogen Metabolism in Milking Cows*

The central problem in the nitrogen nutrition of ruminants is to what extent the microbial flora of the rumen is able to form protein from ammonium nitrogen. A large number of feeding experiments in which a relatively small part of the proteins has been replaced with urea or some other non-protein nitrogen compound have been performed in many countries. They have, however, not given any solution to the above-mentioned problem.

The protein requirement of a cow with a high milk production is exceptionally great, because much more protein is secreted in milk during 24 hours than what is needed for the maintenance of the cow. Thus it is possible to study the extent of the protein synthesis of the microbial flora of the rumen only with dairy cows. This has been the leading idea since the study of milk production was started in this laboratory six years ago with cows fed purified protein-free feed, urea and small amounts of ammonium salts as practically the sole source of nitrogen. Results of these studies have been described partly in numerous papers.[1–8] Oltjen has recently started to study milk production using purified diet.[9]

The following scheme (Fig. 1) describes roughly the nitrogen metabolism of ruminants. The proteins of the feed pass partly without decomposing into the abomasum through the proventriculi, after which their utilization is similar to that in other mammals. Part of the proteins are hydrolyzed in the rumen to amino acids, which will partly be used for microbial protein synthesis, but mainly they will probably be deaminated to ammonia. The digestible non-protein nitrogen of the feed is utilized as ammonia, which the rumen microbes use for their protein synthesis. Part of the ammonia passes into the blood through the wall of the rumen. In the liver some of it will be used for the synthesis of non-essential amino acids, some is metabolized to urea. Urea can be transported from the blood into the rumen, at the same time decomposing into ammonia. The presence of urea in saliva is well known. The surplus of urea is removed in urine. It seems that the ammonia concentration of the rumen regulates to some extent the transfer of urea from the blood to the rumen. The recent experiments by Varady

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*) Lecture given at the 9th Annual Ruminant Nutrition Conference in Atlantic City, U.S.A., on April 15th, 1968.

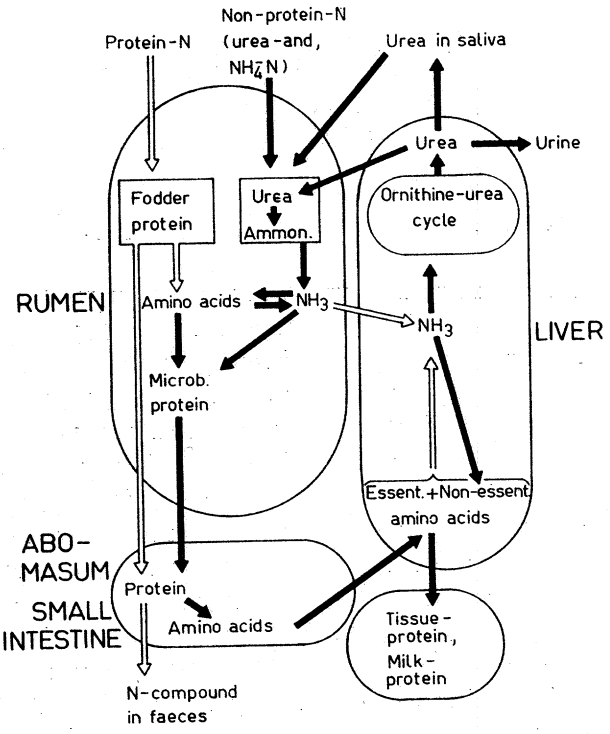


Fig. 1. Scheme of the utilization of protein and non-protein nitrogen in ruminants.

and his colleagues about the effect of starvation on urea retention and on the NH_3 concentration in the rumen of sheep after intravenous administration of urea have confirmed this opinion.[10] As said above our problem has been to elucidate to what extent non-protein nitrogen functions alone without protein nitrogen.

Ten years ago an experiment in which a cow on normal feed was given one dose of ammonium sulfate labeled with ^{15}N was performed in our laboratory. The labeling of the amino acids, separated by fractionation after hydrolysis of the milk proteins, was determined.[11] All the protein amino acids studied were labeled, but the degree of labeling of the various amino acids differed. The weak labeling of some amino acids suggested that their synthesis may form a bottleneck in protein synthesis. Thus the possibility of developing in the rumen, by adaptation to a test feed, a microbial flora which could synthesize protein from ammonia more effectively than the rumen microorganisms of cows on normal feed and thus make milk production possible seemed to deserve experimental study.

The experiments on protein-free feed with urea and small amount of ammonium salts as the sole sources of nitrogen were started with one cow

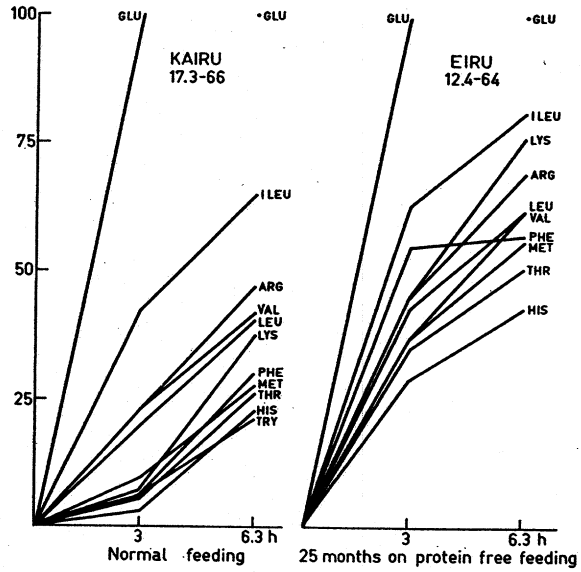


Fig. 2. The ^{15}N labeling of the essential amino acids of the proteins of milk 3 and 6.3 h after the cows had been given a single dose of ^{15}N-ammonium sulfate. The results are given as percentage of the labeling of glutamic acid, since this amino acid is always labeled most rapidly. On the left are the results of a cow on normal feed, on the right those of a cow which had been on test feed for 25 months. Determinations by M. Kreula and T. Moisio.

in the autumn of 1961 and with another at the beginning of 1962. A very slow adaptation procedure was used. When one of the test cows (Eiru) was given a dose of ^{15}N-labeled urea after having been on the test feed for 6 months and the labeling of the amino acids of milk protein was quantitatively determined 6.3 hours and 20 hours after the administration of ^{15}N, the labeling of essential amino acids generally, and especially of some of them, was found to be strongly increased relative to values obtained in experiments with a cow not adapted to the test feed. Another experiment, in which ^{15}N-ammonium sulfate was given the same test cow after it had been on the test feed for 25 months, gave similar results. Accordingly, a dose of ^{15}N-urea and a dose of ^{15}N-ammonium sulfate have the same effect on the labeling of the amino acids of milk protein. The curves in Fig. 2 illustrate the effect of adaptation to the protein-free test feed on the labeling of the essential amino acids of milk protein. The labeling of the different amino acids is expressed as a per cent of the labeling of glutamic acid which always has the highest labeling during the first ten hours.

Four more cows were included in the experiment 1963—64 and later on still two. The adaptation time of 1—2 months seems sufficient, provided that the transferance takes place at the end of the lactation period.

The test cows were fed twice a day. The cows producing greater amounts of milk ate their larger rations gradually all day long and regulated in this way the intake of urea. There was thus no need to divide the feed repeatedly into small portions.

The test feeding was made up of the components as shown in Table 1.

Table 1

Composition of the components of the test feed

1. Composition of the briquettes, about 9 g each:

		1962–64 (%)	1965 (%)
α-Cellulose powder		9.5	9.9
Starch		57.0	52.9
Sucrose		20.9	23.1
Mineral salt mixture		8.2	8.9
Urea (94%) + ammonium salts (6%)	(calc. as urea)	4.4	5.2
	Total	100.0	100.0
	Water content	15.0	15.0

2. Composition of the wet cellulose-rich paste:

		1962–63 (%)	1964– (%)
α-Cellulose powder		60.3	57.3
Starch		19.5	16.4
Sucrose		12.1	12.2
Mineral salt mixture		7.1	8.8
Urea		1.0	5.3
	Total	100	100
Mixed with water.	Water content	75	75

At need, more urea could be mixed with the paste or the sugar-free briquette powder, which was occasionally used.

3. Cellulose strips, 75% water, urea 4.0% + mineral salt mixture 3.0% in dry matter.
4. Vegetable oils, 50–130 g per cow per day.
5. Vitamins A (100 000 IU) and $D_2 + D_3$ (20 000 IU), from 1.1.65 also vitamin E.
6. Mineral salt mixture.

Nitrogen-containing impurities are present in our test feed in such a small amount that the urea and ammonium nitrogen account for 99.5% of the total nitrogen.

Different test cows were given briquettes, cellulose-rich paste, and cellulose strips in different proportions according to appetite. Thus the feed

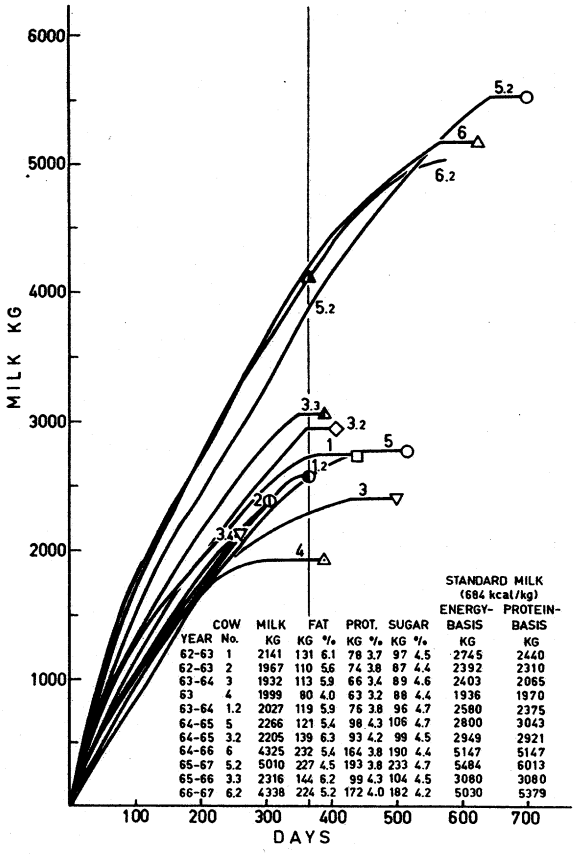
of one cow differed from the feed of another to some extent. Roughly speaking it can be said that the carbohydrate of the test feed was starch, cellulose, and sucrose in the following proportions: 50 to 60% potato starch, 20 to 30% α-cellulose and 17 to 23% sucrose. The success of the urea feeding is decisively dependent on the composition of the carbohydrates fed. The proportion of starch or other rapidly digestible polymer carbohydrates in the feed cannot probably be reduced very much without an accompanying drop in milk production.

As is seen in Table 1 the nitrogen content of the feed has been raised considerably during the experiment. The largest amount of urea (ammonium nitrogen included) during the first 2 years was only a little more than 400 g/day. As investigations on the rumen contents and blood showed, however, that ammonia had not accumulated in the rumen to an injurious degree and that the ammonia content of the blood had not risen alarmingly, the amount of nitrogen in the feed was raised to such an extent that the cows, weighing about 450 kg, could receive even 600—680 g urea per day, corresponding to 1 200—1 360 g dig. crude protein. This addition has raised the milk production very much and it has totally removed those symptoms of probable nitrogen deficiency which were observed when the low-nitrogen feed was used. The hairy coat of the fore part of the legs of the cows, especially that of the hind legs, thinned, or in some cases disappeared, after about 2 months from calving and regrew after the milk production had fallen to 5—7 kg/day. On the basis of our many years experience there is no danger of too high urea rations provided that the feed contains enough carbohydrates of good digestibility.

In order to improve rumination we used rye or wheat straw at first. However, as straw was not included in our program, we tried to omit it by feeding the cows cellulose strips impregnated with silicic acid, which in turn was gradually replaced by polyethylene pellets. Without these pellets the cows ruminated about one hour in 24 hours. 400 g pellets per day approximately doubled the rumination time.

The curves of Fig. 3 show how greatly the milk yield has risen since the amount of urea in the feed was increased in 1964 and 1965. So far, the highest production of standard milk per year has been on an energy basis 4 217 kg, and on a protein basis 4 284 kg; the highest production for a prolonged lactation period has been on an energy basis 5 484 kg, and on a protein basis 6 013 kg (cow No. 5). We have used as standard milk, milk containing 4.0% fat, 3.2% protein and 4.9% sugar (684 kcal/kg milk).

The daily intake of test feed was at most 11 kg dry substance corresponding to nearly 10 fu. A daily production of about 17—18 kg standard milk is thus theoretically possible. This is mostly reached, and often exceeded, when body reserves are partly used for milk production.



YEAR	COW No.	MILK KG	FAT KG	FAT %	PROT. KG	PROT. %	SUGAR KG	SUGAR %	STANDARD MILK (684 kcal/kg) ENERGY-BASIS KG	STANDARD MILK (684 kcal/kg) PROTEIN-BASIS KG
62-63	1	2141	131	6.1	78	3.7	97	4.5	2745	2440
62-63	2	1967	110	5.6	74	3.8	87	4.4	2392	2310
63-64	3	1932	113	5.9	66	3.4	89	4.6	2403	2065
63	4	1999	80	4.0	63	3.2	88	4.4	1936	1970
63-64	1.2	2027	119	5.9	76	3.8	96	4.7	2580	2375
64-65	5	2266	121	5.4	98	4.3	106	4.7	2800	3043
64-65	3.2	2205	139	6.3	93	4.2	99	4.5	2949	2921
64-66	6	4325	232	5.4	164	3.8	190	4.4	5147	5147
65-67	5.2	5010	227	4.5	193	3.8	233	4.7	5484	6013
65-66	3.3	2316	144	6.2	99	4.3	104	4.5	3080	3080
66-67	6.2	4338	224	5.2	172	4.0	182	4.2	5030	5379

Fig. 3. Milk production of test cows on experimental feed. The milk yield was calculated both on the basis of energy (standard milk, 684 kilocalories per kilogram of milk) and of protein (standard milk, 3.2 percent protein). The large numbers on the curves refer to the test cows, the small numbers to the lactation periods during the experimental feeding (for example, 3 = cow No. 3, first period; 3.2 = cow No. 3, second period); the end points of the curves represent the calving times. The annual milk yields may be seen from the curves. Notice the very similar milk production of cow No. 6 (Metta 12 years old 1967) during two successive lactation periods.

It is characteristic of high yielding test cows that they have become pregnant only after several services. The estrus of all the test cows has been regular and the reason for the delay in fertilization of the high-producing test cows is so far unknown. The addition of vitamin E to the test feed has not improved the fertilization, according to the results so far.

The calves have been strong and vigorous, weighing on the average 36 kg. They grow steadily on purified feed with urea as the sole source of nitrogen since the fifth month, reaching the weight of 250—290 kg when one year old.

On the basis of our results the heifers did not, however, develop into cows on such a feed, because they died suddenly when 1—2 years old. It is only since the use of α-tocopherol has been started that we have managed to rear milk cows from heifers.

The observations on the rapid utilization of ammonia in the rumen of the cows adapted to the test feed could be explained only by supposing that the microbial flora of the rumen content had been effectively adapted to the utilization of ammonium nitrogen. Investigations in our laboratory concerning the microbial flora of the rumen have shown that the protozoa in the rumen of the adapted cows have generally disappeared, while the number of bacteria has risen enormously (Fig. 4 and Table 2).

It is probable that the bacterial strains which grow well with NH_4-N without amino acids, vitamins, or other specific growth factors are the most important in the bacterial flora of the rumen of the test cows. There are, however, in the ruminal flora even bacteria which need specific growth factors for their growth. These can be important for the effective function of the rumen. Regarding symbiotic growth, I refer to the fundamental work of Nurmikko in our laboratory on the lactic acid bacteria 15 years ago.[12]

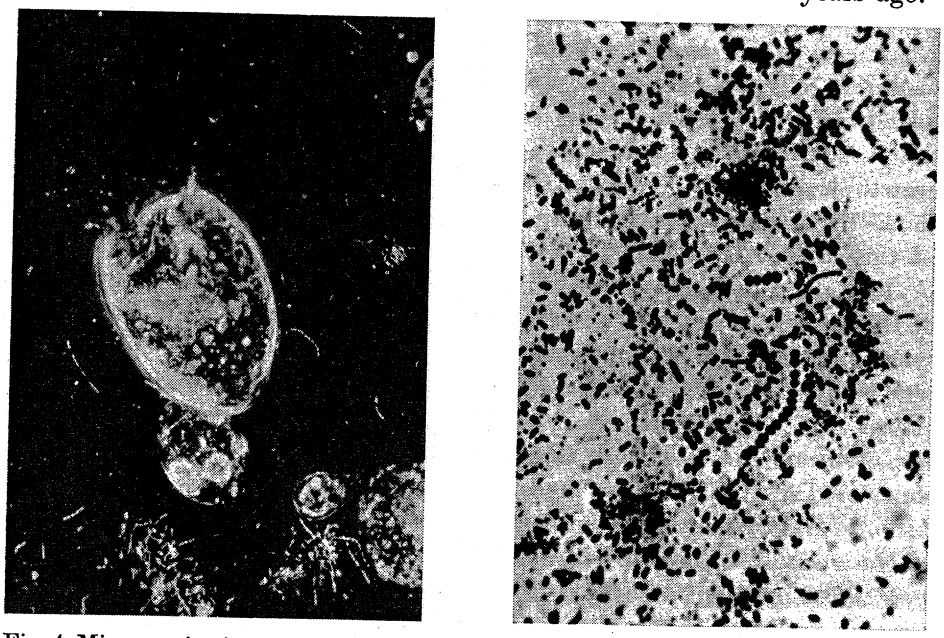


Fig. 4. Microscopic pictures of the rumen contents of cows on normal feed and test feed. Left-hand figure: normal feeding. Plenty of big and small protozoas besides bacteria. ×430. Photo M. Lampila.

Right-hand figure: test feeding. Protozoa were not observed microscopically with low magnification. In the picture masses of bacteria of different types. ×1000. Photo S. Mäkinen.

Table 2

Microbial counts, urea and ammonia concentrations found in the rumen contents of test cows and normally fed cows.

	Time of sampling	Protozoa per ml	Bacteria per ml	Urea mg/100 ml	Ammonia mg/100 ml
Test cows:					
Metta (No. 6)	10 min after feeding	0	3.1×10^{10}	17.5	32.9
—»—	100 min after feeding	0	4.3×10^{10}	0.4	4.3
Jairu (No. 5)	3 h after feeding	0	3.0×10^{10}	13.4	9.0
—»—	7 h after feeding	0	1.2×10^{10}	2.6	1.6
Normally fed cows:					
Asteri	3 h after feeding	7.9×10^{4}	3.6×10^{9}	0	22.4
Mili	—»—	4.4×10^{4}	2.3×10^{9}	0	16.6
Muoti	—»—	1.0×10^{5}	3.7×10^{9}	0	22.7

He showed in his dissertation conclusively that different strains of lactic acid bacteria can feed one another with different growth factors when they are grown in the same medium, because the factors synthesized in the bacterial cells are partly excreted into the medium. This means that in mixed cultures different strains can grow in a medium that is much simpler than that needed by the same strains in pure cultures (Fig. 5). The symbiotic growth is probably general in microbial associations, and the microbial flora of the rumen contents may be one of the most complicated examples of this type of system.

In the amino acid composition of the hydrolyzate of the total protein of the rumen contents of test cows and of control cows on normal feed, systematic differences could clearly be found only regarding α, ε-diaminopimelic acid, a characteristic constituent of the cell wall of many bacteria. It was regularly present in much higher amounts in the hydrolyzate of the rumen protein of test cows than in that of normally fed cows. There is some evidence also for the higher concentration of γ-amino butyric acid and ornithine, both components of the bacterial cell wall, in the hydrolyzate of the rumen protein of test cows.

Studies on the composition of the nitrogenous compounds of the rumen contents have shown that the ammonia in the rumen does not increase to values which should be harmful; besides, it falls rapidly after feeding (Fig. 6) and is then lower than on normal feeding. The highest amount of ammonia we have found in the rumen contents of test cows just after the feeding, has been not quite 40 mg/100 ml. During the highest and most rapid intake of feed the amount of ammonia will probably rise greater than this.

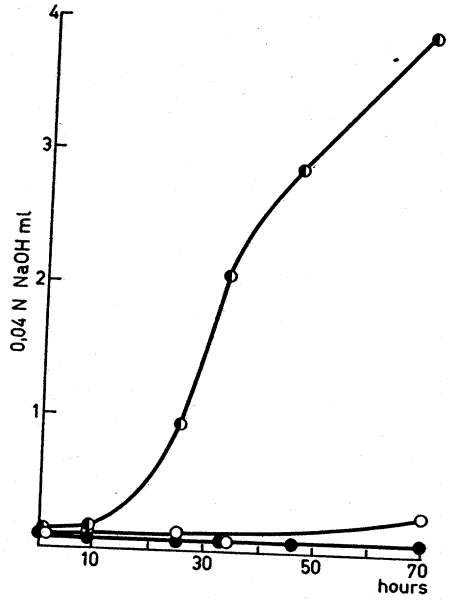


Fig. 5. Growth of *Lactobacillus arabinosus* 17-5 and *Streptococcus faecalis* R in symbiosis.
Basal medium without phenylalanine and folic acid.
● *Lb. arabinosus* 17-5 (phenylalanine-requiring strain).
○ *Str. faecalis* R (folic acid-requiring strain).
◐ *Lb. arabinosus* 17-5 and *Str. faecalis* R together.

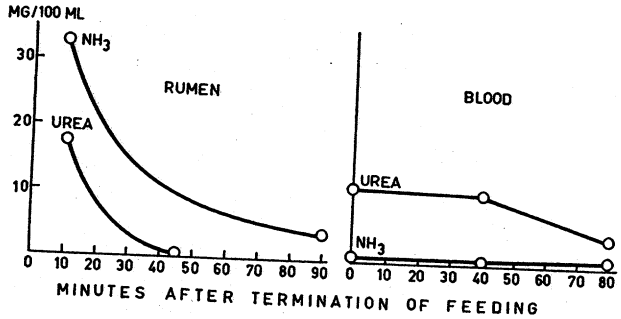


Fig. 6. Ammonia and urea content in the rumen and blood of test cows.

The composition of the nitrogenous compounds of the feces of the test cows has also been studied in our laboratory. In experiments with rats, McNaught *et al.*[13] found the digestion coefficient of the protein in a bacterial fraction grown in strained rumen liquor with urea and carbohydrate supplement to be 74. If this coefficient is the same in ruminants, the main part of the nitrogen of the feces of the test cows should be indigestible bacterial protein. In fact, among the normal amino acids which are found

Table 3

Analyses of some nitrogen compounds in the urine of cows.

	Total N %	NH_4-N %/tot. N	Urea-N %/tot. N	Creatinine-N %/tot. N
Normal feeding (Farm 1)	1.13	3.0	30.3	6.0
Normal feeding (Farm 2)	1.27	0.6	33.2	4.4
Protein-free test feeding	0.48	2.1	49.6	6.8
Low protein, high urea feeding	0.72	0.7	40.3	5.8

in the hydrolyzate of the protein fraction of the feces of the test cows, α, ε-diaminopimelic acid is present in much higher amounts than in the same fraction from cows on normal feed.[6] This is in agreement with findings for the amino acid composition of the proteins of the rumen contents. Although not all rumen bacteria contain diaminopimelic acid in their cell wall »proteins», the high content of this amino acid in the protein fraction of feces strongly supports the implication of other results, that bacterial protein synthesis is greatly enhanced in the rumen of the test cows. The ammonia, urea and creatinine nitrogen in urine is seen in table 3.

Because the mammary gland receives the raw material for the synthesis of different components of milk from the blood, knowledge of the composition of the blood of the test cows is important.

The free amino acids of the blood form only a very small part of the nitrogenous compounds of the blood, but they are decisively important for the formation of the proteins of milk in the mammary gland. The concentrations of most of the free amino acids, particularly the essential amino acids, in the plasma and the whole blood of the lactating test cows are lower than those in plasma and blood of the control cows on normal feed (Fig. 7). The relative decrease in the concentration of free histidine in plasma is greater, in cows on the test feed, than the decrease for any other amino acid. The histidine content decreases some weeks after calving, remains low for several months, and then rises again to some extent while the milk production is decreasing. At its lowest level, the free histidine content of the plasma can be about 20 percent of the corresponding values for normally fed cows.

Low concentrations of free amino acids are found in the plasma of the lactating test cows; the concentrations of histidine and many other amino acids in the plasma of dry test cows and of heifers are much higher.

The very low ammonia concentration in the blood of the test cows is in accordance with the great ability of the adapted ruminal flora to utilize ammonium nitrogen. Whether the ability of the liver to utilize ammonium

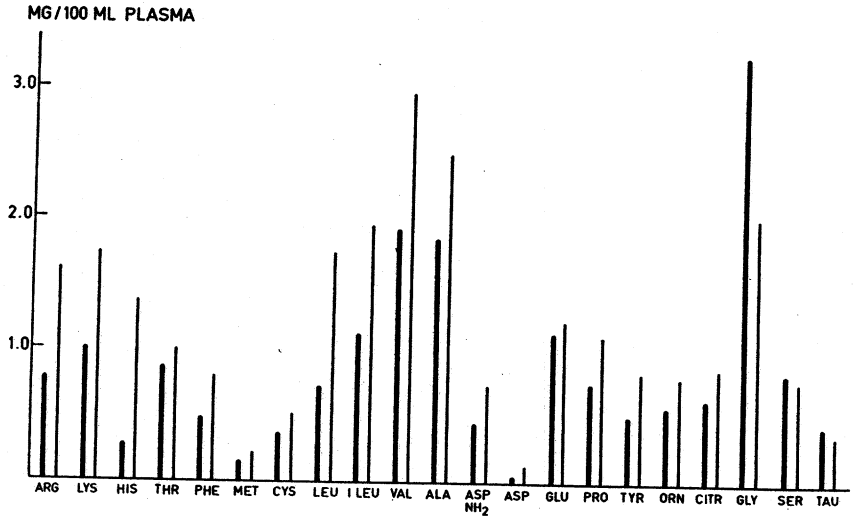


Fig. 7. The amount of free amino acids (mg/100 ml) in the blood plasma of milk-producing cows.
Thick columns: test cows fed on purified protein-free feed.
Thin columns: cows on normal feed.

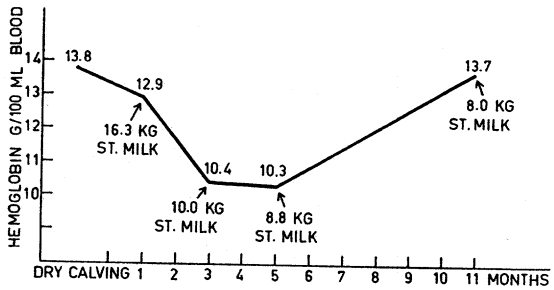


Fig. 8. Hemoglobin content of the blood of test cow No. 5 (Jairu) 1, 3, 5 and 11 months after calving.

nitrogen has increased is not known but this is possible. Holzschuh and Wetterau have recently considered this to be the case.

The hemoglobin content of the blood of the test cows decreases during the highest milk production more than that of normally fed cows (Fig. 8). It seems, however, that the difference between the test cows and normally fed cows is smaller when more urea is used in the feed. Since the content of free histidine in the plasma of the test cows is very low, and histidine may form a bottleneck in protein synthesis in cows on the test feed, it may be that the primary reason for this reduction in hemoglobin is the deficiency of histidine during the post-calving period, when the protein requirement is greatly increased because of milk production.

The amino acid composition of the whole-blood protein of the test cows was similar to that of normally fed control cows. In electrophoretic studies carried out in this laboratory on the serum proteins of several blood samples of the test and control cows, only slight differences in the various protein fractions in samples from individual cows could be observed, and the differences in the results for test and control cows were slight.

Milk produced on a protein-free feed is called zero milk (0-milk) in our laboratory. Its composition has been the object of many-sided research. We have been especially interested in comparing the proteins of the 0-milk with those of milk produced on normal feed.[14] All the methods we have used — DEAE-filtration and various gelelectrophoreses — have led to the result that no certain differences due to the feed have been observed. (Figures 9, 10, 11.)

According to Larson and Gillespie over 90% of the total protein in milk is synthesized in the mammary secretory cells from free amino-acids.[15] The synthesis of these proteins is carefully controlled by the genes. If some amino acids are not present in sufficient amount, protein synthesis decreases. The second small group including the immune globulins are according to the authors blood proteins entering milk from the blood stream.

The flavor of 0-milk is to such an extent similar to that of normal milk that it is not easy to distinguish between these milks in organoleptic tests, provided no off-flavor due to the feed used occurs in normal milk. I have been especially interested in this problem, because it has not earlier been known to what extent milk flavor is due to the feed and to what extent the flavor substances of milk are formed in the cow's organism. The flavor of 0-milk and normal milk has been studied gaschromatographically and mass-spectrometrically in our laboratory for many years.[5] The compounds having a major role in milk flavor are found both in 0-milk and normal milk.

When basic knowledge about the milk production of cows on purified, protein-free feed had been obtained, there arose the question, what the

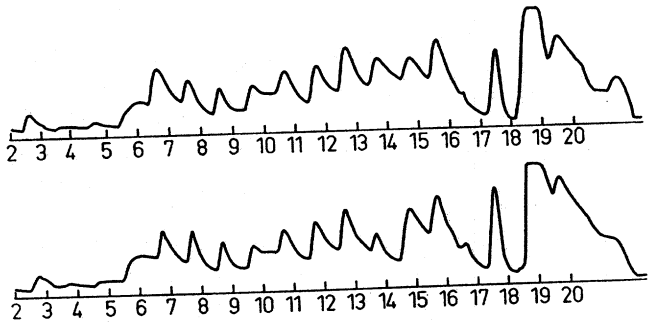


Fig. 9. Fractionation of the milk proteins on a DEAE-column.
Above: milk of the test cow Eiru (No. 1), below: milk of cows on normal feeding.

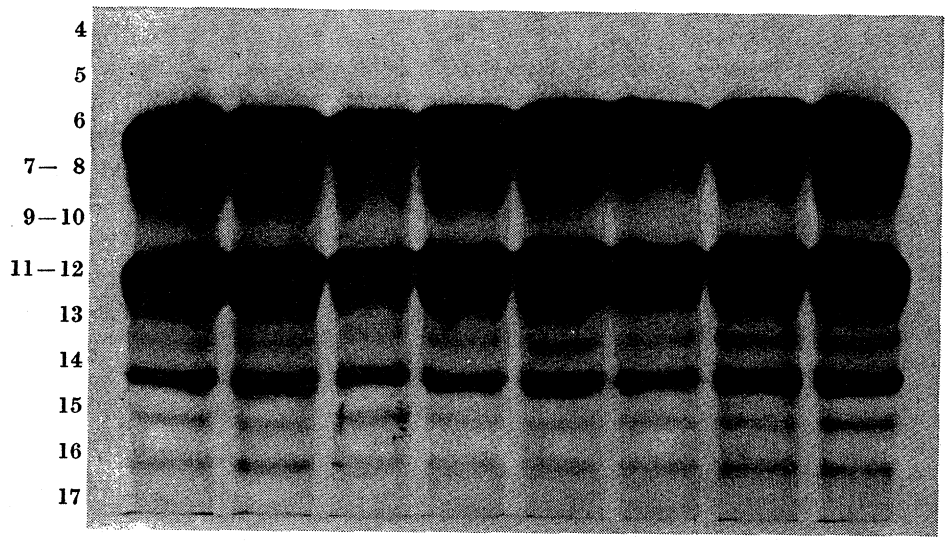


Fig. 10. Starch gel electrophoresis of acid caseins from milks of the test cows Aino (A), Metta (M), Jairu (J), Eiru (E) and control milk I (C_1). The samples were taken in December 1964. Zone designations are according to Neelin.[16]

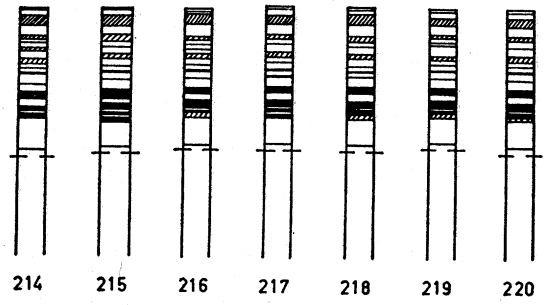


Fig. 11. Drawings of disc electrophoretic patterns of the whey proteins of the test cows, Aino (214, 218), Jairu (215), Metta (216, 219) and control milk I (217, 220). The samples in tubes 214—217 were taken in February and in tubes 218—220 in April 1965.

results would be if non-purified feed containing different amounts of true protein would form the feed of cows. The comparison of feed low in digestible true protein supplemented with large doses of urea was of greatest interest. A feeding experiment using rations containing about 20, 40 and 50% digestible true protein of the digestible crude protein was arranged. To reach such a low content of digestible true protein in the feed as 20% is difficult. We used in the daily ration fresh potatoes, dried sugar beet pulp and dried

Table 4

Feeding during the highest milk production/day.

Feed		Cow No.						
		20.1	20.2	21.1	22.1	23.1	24.1	25.1
Potatoes, kg	(d.m. 20%)	22.5	20.0	—	—	—	—	—
Sugar beet pulp, kg	(d.m. 90%)	3.5	7.0	4.7	4.7	—	—	—
Oats, kg	(d.m. 90%)	—	—	5.5	5.0	6.0	8.5	6.0
Barley, kg	(d.m. 90%)	—	—	1.5	2.0	4.0	2.0	1.5
Hemicellulose, kg	(d.m. 96%)	2.3	3.0	3.0	3.0	3.0	3.0	3.0
Silage, kg	(d.m. 20%)	—	—	—	—	—	—	16.0
Hay, kg	(d.m. 80%)	—	—	—	—	—	—	2.0
Straw, kg	(d.m. 80%)	1.5	0.3	1.0	1.0	2.0	2.0	—
0-fibre, kg	(d.m. 100%)	2.3[1]	1.0[2]	0.4[1]	—	—	—	—
Urea, g		440	560	440	440	350	370	340
Salt mixture, g		750	750	400	400	400	400	350
Feed units/day		10.2	12.9	12.8	12.7	12.3	12.4	12.3
Feed units/year		3365	~3750	3583	3875	3026	3549	3811
Urea, kg/year		136.0	155.0	123.1	129.3	93.9	95.1	89.0

[1]) 0-fibre of low quality, digestibility about 50%.
[2]) 0-fibre of high quality, digestibility 80%.

hemicellulose syrup prepared from wood. Digestible urea nitrogen formed in this diet 65% of the digestible crude protein. It was already easier to arrange the feed rations containing 40% or more digestible true protein of the digestible crude protein, because oats and barley could then be included in the feed rations. Table 4 shows the composition of the daily feed ration of cows No. 20—25 during the period of high milk production after calving. 20.1 and 20.2 mean cow No. 20 after the first and second calving, respectively. In Fig. 12 the annual milk yields which have been obtained using the feed combinations described in table have been shown with columns. For comparison the figure also contains the best annual milk yields of the test cows fed on protein-free purified nutrients, with urea and ammonium salts as the sole sources of nitrogen.

The figure shows clearly that when non-purified feed low in digestible true protein but supplemented with high doses of urea has been used, milk production has risen considerably compared with the milk production of cows on protein-free feed. The increase has been roughly 1 000 to 1 500 kg milk per year when the feed contained 20% and 40% digestible true protein respectively. This is due to the higher intake (2—3 feed units) of non-purified feed containing some protein. The amount of feed eaten depends again obviously on the utilization of the feed in the rumen and other parts

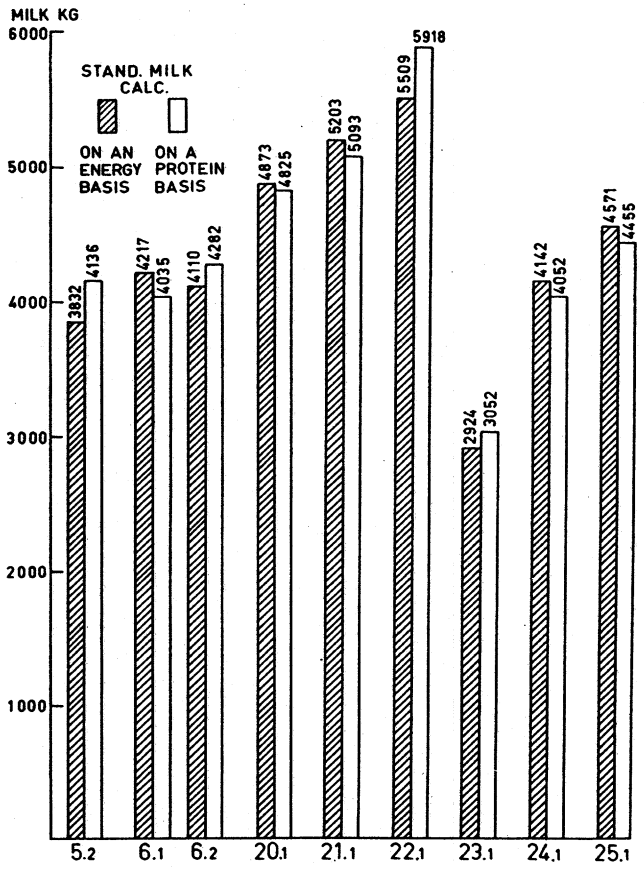


Fig. 12. Milk production of test cows on a protein-free feed (cows No. 5 and 6) and of cows on feed containing about 20, 40 and 50% digestible true protein (cows No. 20—25).

	Cow No.	Urea fed/year	Feed units (fu)/year	Dig. true protein % of dig. crude protein
Purified rations without protein	5.2	169.7 kg	2695	—
	6.1	160.1 »	2838	—
	6.2	189.3 »	2798	—
Feed with different protein content	20.1	136.0 kg	3365	21.1%
	20.2	~164.7 »	~3928	~21.2 »
	21.1	123.1 »	3583	39.7 »
	22.1	129.3 »	3875	40.5 »
	23.1	93.9 »	3026	44.6 »
	24.1	95.1 »	3549	48.7 »
	25.1	89.0 »	3811	50.5 »

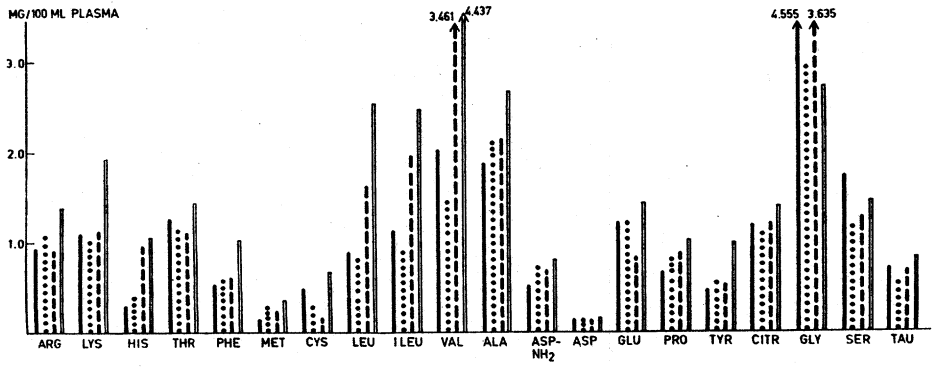


Fig. 13. The amount of free amino acids in blood plasma. Protein-free feed (dark columns), 20% dig. true protein of dig. crude protein (dotted line columns), 40% dig. true protein of dig. crude protein (broken line columns), normal protein-rich feed (white columns).

of the intestinal canal. As non-purified feed contains a great number of most different compounds which are not found in purified nutrients, it is difficult to say what substances in the non-purified feed have increased the intake of fodder. On the basis of blood analyses digestible true protein increases the amount of free amino acids in the plasma when digestible true protein is included in the feed (Fig. 13). The sister cows 21 and 22 on a feed containing about 40% dig. true protein-N and 53% dig. urea-N of the dig. total-N have given the highest annual milk yields. During the second year the production of cow 22 is expected to reach 7 000 kg standard milk. Cows No. 23 and 24, the feed rations of which have contained 45—49% dig. true protein-N of the dig. total protein-N and correspondingly less urea-N, have given lower milk yields due partly to a lower milk production capacity, partly owing to a less suitable ration composition (more grain, no sugar beet pulp). As the number of cows in our feeding experiments has been small, the real comparison between milk yields is of course questionable. As said before, also other groups of compounds than true protein may influence the rise in milk production when non-purified feed is used. We have found for instance in the blood serum of our test cows on purified, protein-free feed on the average only about 50 mg% cholesterol and 160 mg% total lipid, the corresponding values on normal feed and on low-protein experimental feed being three times higher. As far as I know such low cholesterol and lipid contents have been found with no other adult animal as with our test cows on protein-free feed.

Photos of cows No. 6 and 20 are presented in Figures 14 and 15.

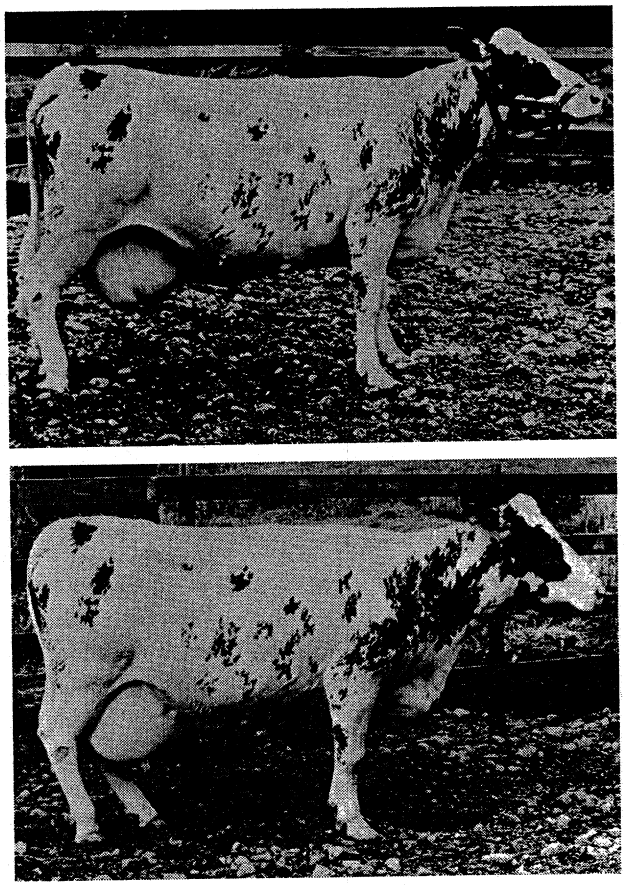


Fig. 14. Test cow Metta (No. 6) after being on the purified, protein-free test feed for 1 year (at the top) and for 3 years (below). Age of the cow in the latter case 12 years.

It is clear that experiments with a few cows only show the possibilities for dairy cows to utilize urea. Large scale experiments for comparison of the effectiveness of protein and urea as nitrogen sources for dairy cows are needed using different carbohydrate sources. In any case, our experiments have shown that it is possible to reach a surprisingly high milk production, using feed low in protein and rich in urea.

Large amounts of hemicellulose syrup prepared from wood have been used in our experiments of a practical nature, because we wanted to elucidate the usability of this quantitatively important component of wood in the feed of dairy cows. As the protein can be substituted by urea in such amounts which earlier seemed impossible the central problem in the feeding of dairy cows is how to provide enough carbohydrates with high digesti-

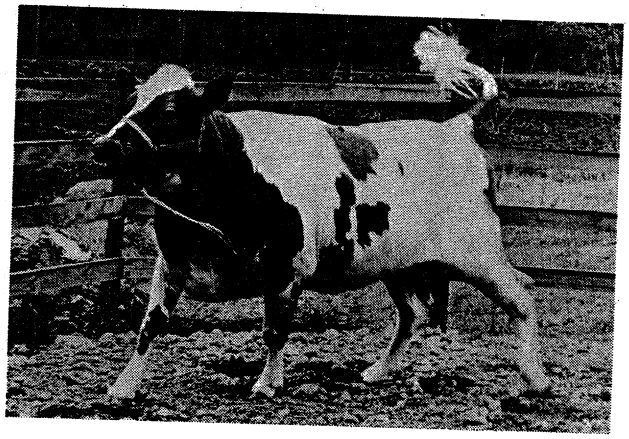


Fig. 15. Heifer-cow Lila after being on a feed poor in protein and rich in urea for 16 months.

bility. In tropical countries sugar cane molasses, sweet potatoes and similar plants rich in starch give possibilities for considerable milk production.

After this lecture was given I received a manuscript of H. R. Conrad, J. W. Hibbs and J. R. Staubus from Ohio Agricultural Research and Development Center, Department of Dairy Science, Wooster: »Guidelines for Increasing Urea Utilization in Rations for Dairy Cows», in which successful experiments with dairy cows are described when large amounts of urea are used.

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